Claims

1. A method for loading paramagnetic particles consisting of a core matrix containing paramagnetic material and having stably attached to its surface activatable functional groups capable of forming a chemical bond with nucleophilic groups on antibodies or antibody fragments, with antibodies or antibody fragments, which method comprises reacting particles having activated functional groups with antibodies or antibody fragments, and subsequently completely inactivating the remaining activated functional groups.

2. The method according to claim 1, wherein the core matrix essentially consists of a matrix material selected from the group consisting of silica, aluminum hydroxide, hydroxyapatite and zirconium hydroxide.

3. The method according to claim 1, wherein the paramagnetic material is selected from the group consisting of $MnSO_4$, $FeSO_4$, $CoCl_2$ and $NiSO_4$.

4. The method according to claim 1, wherein the activatable functional groups capable of forming a chemical bond with nucleophilic groups on the antibodies or antibody fragments are selected from the group consisting of directly activatable functional groups and functional groups activatable through reaction with a bifunctional reagent.

5. The method according to claim 4, wherein said activatable functional groups are selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, -SH, CHO, -OH, acetals, epoxy groups or activated derivatives thereof.

6. The method according to claim 5, wherein the functional groups are -COOH groups or activated derivatives thereof.

7. The method according to claim 1, wherein the activatable functional groups are attached to the surface of the paramagnetic core matrix via a linker molecule having the activatable functional group at its terminal end.

8. The method according to claim 7, wherein the linker molecule comprises the structure –X-tg, wherein X is a C_{1-20} group optionally interrupted by one or more heteroatoms and tg is a functional group as defined hereinbefore.

9. The method according to claim 1, wherein the activatable functional groups are attached to the surface of the paramagnetic core matrix via a coating with a natural or synthetic polymer carrying the activatable functional groups.

10. The method according to claim 9, wherein the natural or synthetic polymer is selected from the group consisting of homopolymers or copolymers derived from monomers having unsaturated carbon chain and a functional group as defined hereinbefore, or a protected form thereof.

11. The method according to claim 9, wherein the monomer is acrylic acid or a derivative thereof.

12. The method according to claim 1, wherein the activatable functional group is a –COOH group attached to the surface of the particle via a linker molecule, the activation is effected by treatment with a carbodiimide and N-hydroxy succinimide and the inactivation of remaining activated –COOH groups is effected by treatment with a mono - or - di C_{1-4} alkylamine which might be substituted by polar groups.

13. The method according to claim 12, wherein the alkylamine is ethanolamine and the treatment is performed at pH 7.40 to 7.45.

14. A method for isolating and identifying specific target cells contained in body fluids, which comprises the steps of

(a) (1) mixing paramagnetic particles loaded with first antibodies/antibody fragments directed against the target-cell specific membrane structures, or mixtures of said first antibodies/antibody fragments according to the method defined in claim 1 with the body fluid containing the target-cells, or

(2) mixing and incubating free first antibodies/antibody fragments or mixtures of said first antibodies/antibody fragments with the body fluid containing the target cells;

(b) (1) incubating the mixture obtained in step (a1), or

(2) mixing and incubating the mixture obtained in step (a2) with the paramagnetic particles loaded with second antibodies/antibody fragments capable of specifically binding to said first antibodies/antibody fragments according to the method defined in claim 1; and

(c) subjecting the mixture obtained in step (b) to a magnetic field to therewith separate the specific target cells from the mixture,

provided that steps (a) and (b) do not encompass a pre-incubation with amphiphiles.

15. The method of claim 14, wherein the body fluid prior to its mixing with the loaded particles or first antibodies/antibody fragments is subjected to dilution or ammonium chloride lysis.

16. The method of claim 15, wherein said lysis is performed by adding a solution containing NH_4Cl, $KHCO_3$ and EDTA.

17. The method of claim 14, wherein prior to or after step (c) the reaction mixture is incubated with third antibodies or antibody fragments labeled with functional moieties permitting their visualization by a chemical or physical reaction, said third antibodies/antibody fragments being directed

to extracellular or intracellular molecules present in the target cells, but differing from the membrane structures recognized by the first antibodies/antibody fragments.

18. The method of claim 17, wherein said third antibodies or antibody fragments are labeled with enzymes.

19. The method of claim 14, wherein the first or second antibodies/antibody fragments or the beads are labeled with functional moieties permitting their visualization by a chemical or physical reaction.

20. The method of claim 14, wherein after step (c) a counting of the stained or unstained particle-cell-complexes in the cell suspension is performed using a microscope and/or a suitable cell/particle counting device.

21. The method according to claim 14, wherein the body fluids are derived from sources selected from the group consisting of peripheral blood, bone marrow aspirates, pleural or peritoneal effusions, urine, cerebrospinal fluid, semen, lymph, solid tumors.

22. The method according to claim 21, wherein the body fluids are derived from sources selected from the group consisting of peripheral blood and bone marrow aspirates.

23. The method according to claim 21, wherein the body fluids are of human origin.

24. The method according to claim 14, wherein the specific target cells are primary abnormal cells selected from the group consisting of tumor cells, metastatic tumor cells and disseminated tumor cells.

25. The method according to claim 24, wherein the specific target cells are selected from the group consisting of cells of breast cancer, ovarian cancer, lung carcinoma, melanoma, sarcoma, glioblastoma and other cancers.

26. The method according to claim 14, wherein said first and second antibodies/antibody fragments are selected from the group consisting of the IgG, IgM and IgA, isotype.

27. The method according to claim 26, wherein the antibodies/antibody fragments are derived organisms like mouse, rat, rabbit, goat, bacteria or phages.

28. The method according to claim 14, wherein said first antibodies/antibody fragments are monoclonal antibodies/antibody fragments

29. The method according to claim 28, wherein the first antibodies/antibody fragments are directed against groups of antigen determinants on the target cells.

30. The method according to claim 29, wherein said antigen determinants are selected from the group consisting of epithelial surface antigen (ESA), Her2/neu, melanocyte cell surface antigen and CD146.

31. The method according to claim 14, wherein said second antibodies/antibody fragments are polyclonal or monoclonal iodotypic antibodies/antibody fragments.

32. The method according to claim 31, wherein the second antibodies/antibody fragments are selected from the group consisting of anti-mouse, anti-rat, anti-rabbit and anti-goat antibodies or antibody

fragments thereof directed against the first antibodies/antibody fragments.

33. The method according to claim 14, which further comprises a wash step of the magnetic particles separated in step (c).

34. The method according to claim 14, which further comprises a step of examining the isolated cells by biochemical, molecular biological or immunological methods.

35. The method according to claim 14, wherein said step of examining includes a characterization of specific genes by identifying nucleic acids and proteins, and elucidating the structure and function of nucleic acids and proteins.

36. The method according to claim 14, which further comprises a step of establishing a culture of the isolated target cells or their complexes with the coated magnetic particles.

37. The method according to claim 14, wherein the incubation step (b) is performed in an incubation buffer containing sugars, citric acid or a salt thereof and lipids.

38. The method according to claim 37, wherein said incubation buffer comprises citric acid or a sodium or potassium salt thereof at a concentration of 2 to 20 mM, a mixture of hexoses and/or pentoses in a total concentration of 5 to 50 mM and lipids in a concentration of 0.01 to 10 g/l.

39 The method according to claim 14, wherein the incubation is performed at 0 to 37°C for 5 min to 2 h under gentle agitation.

40. Paramagnetic particles loaded with antibodies or antibody fragments according to the method of claim 1.

41. An incubation buffer containing sugars, citric acid or a salt thereof and lipids.

42. The incubation buffer of claim 41 which comprises citric acid or a sodium or potassium salt thereof at a concentration of 2 to 20 mM, a mixture of hexoses and/or pentoses in a total concentration of 5 to 50 mM and lipids in a concentration of 0.01 to 10 g/l.

43. A kit for immunomagnetic isolation comprising the loaded paramagnetic particles of claim 40.

44. The kit of claim 43 which is suitable for performing the method according to claim 14 and which comprises

(i) paramagnetic particles loaded with first antibody/antibody fragments as defined in claim 14, or paramagnetic particles loaded with second antibodies/antibody fragments and free first antibodies/antibody fragments as defined in claim 14; and

(ii) an incubation buffer containing sugars, citric acid or a salt thereof and lipids.

45. The kit of claim 44, wherein the incubation buffer comprises citric acid or a sodium or potassium salt thereof at a concentration of 2 to 20 mM, a mixture of hexoses and/or pentoses in a total concentration of 5 to 50 mM and lipids in a concentration of 0.01 to 10 g/l.

46. The kit of claim 44 which further comprises additional components selected from the group consisting of

(iii) solutions and/or salts necessary for the lysis of erythrocytes in whole blood samples;

(iv) wash solutions for washing the cells during separation; and/or

(v) a magnet; and

(vi) target-cell specific antibodies/antibody-fragments differing from the first antibodies/antibody fragments labeled with specific color detectable enzymes, such as peroxidase and alkaline phosphatase.